

The University of Chicago

EFFECT OF VARIOUS GROWTH REGU-
LATING SUBSTANCES UPON SEV-
ERAL SPECIES OF PLANTS

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY

1941

By

DAPHNE B. SWARTZ

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EFFECT OF VARIOUS GROWTH-REGULATING SUBSTANCES UPON SEVERAL SPECIES OF PLANTS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 535

DAPHNE B. SWARTZ

Introduction

Many recent investigations have been concerned with attempts to determine the functions of certain of the phytohormones. Notable among these substances are those—such as vitamin B₁ and nicotinic acid—which have been shown, in experiments with isolated embryos and excised roots, to be essential to root development (1, 8). These experiments indicate also an interdependence of these two substances. For example, BONNER (1, 2) found that while in certain instances nicotinic acid alone was not effective, when applied in addition to vitamin B₁ it intensified the response of the plant to the latter.

But whether these substances, under certain conditions, may become limiting factors to the extent that their external application will affect the total plant growth, is still a question. BONNER and GREEN (3) have reported increased dry weight of both roots and shoots, accompanying increased general vigor, when vitamin B₁ was supplied with nutrient solution to plants in sand cultures. In marked contrast to these results are those reported in the recent work of HAMNER (6). Under variously controlled conditions of photoperiod and nitrogen supply, plants supplied with vitamin B₁ showed no increased dry weight. GORHAM (5) also reports no increased frond multiplication in *Lemna* when vitamin B₁ was added to the solution in which it was growing.

Other substances, such as naphthaleneacetic acid and related synthetic compounds, though not yet known to be formed in plants, are known to increase the rate of root initiation when applied to stems, leaves, and plant parts other than roots, in general (9). These same substances, which initiate root formation, may fail to promote growth and even in high dilutions inhibit root growth (4). Somewhat at variance with this is the report of GORHAM (5) that concentrations of 0.25 and 0.10 mg. of naphthaleneacetic acid per 100 ml. of nutrient produced marked increase in the rate of frond multiplication in *Lemna*. It has also been well established that pronounced histological changes accompany root formation and other external changes induced by naphthaleneacetic acid (7). But the effect which these root-forming substances may have upon total plant growth (as indicated by dry weight), and the external conditions under which these effects may occur, are as yet largely undertermined.

The present investigation, except for a part of the preliminary experiment, attempted only to determine the effects resulting from the addition of various growth substances to plants supplied with a complete nutrient solution to the sand medium in which they were grown. Great numbers of plants were used to make statistical analysis of the results possible.

Investigation

CHRYSANTHEMUM

This experiment was conducted, in part, to determine whether the addition of a growth substance may in any measure compensate for the lack of an essential element in the nutrient solution.

On April 26, chrysanthemum cuttings which had been rooted and were of approximately uniform size were transplanted into 4-inch glazed pots with side drain-

TABLE 1
DRY WEIGHT* IN GRAMS OF CHRYSANTHEMUM PLANTS

	I COMPLETE NUTRIENT		II MINUS Mg NUTRIENT		III MINUS K NUTRIENT		IV MINUS P NUTRIENT	
	TOPS	ROOTS	TOPS	ROOTS	TOPS	ROOTS	TOPS	ROOTS
Control.....	4.72	2.11	0.81	0.17	0.4	0.16	0.17	0.21
Vitamin B ₁ added.....	4.59	2.03	0.95	0.24	0.55	0.28	0.15	0.21
Naphthaleneacetic acid added.....	4.21	1.94	0.72	0.20	0.8	0.39	0.16	0.31

* Weights represent mean of fifteen plants grown under each treatment.

age. The pots had been previously filled with quartz sand and flushed with distilled water. They were supported on lath racks to prevent any contamination of the nutrient supply through drainage. Fifteen plants were provided for each of the four treatments (table 1), and the pots were placed at random on the greenhouse bench in rows of five each. During the 10 weeks which followed, the plants were supplied with nutrient solution, with added growth substances on alternate days, and with distilled water on intervening days. Once a week the pots were thoroughly flushed with distilled water. The complete nutrient solution used in this, as well as in the more extensive experiments which followed, was made up from the following 0.5M stock solutions. Of these there were added to 72 liters of distilled water: 864 ml. of $\text{Ca}(\text{NO}_3)_2$, 648 ml. of MgSO_4 , and 648 ml. of KH_2PO_4 . In the "minus" solutions, the following substitutions were made: NaH_2PO_4 for KH_2PO_4 in the minus K solution, KCl for KH_2PO_4 in the minus P solution, and Na_2SO_4 for MgSO_4 in the minus Mg solution. Minor elements were supplied in

the following parts per million of nutrient: boron 0.5, manganese 0.5, zinc 0.05, and copper 0.02. A 0.5 per cent iron tartrate solution was added once weekly at the rate of 1 ml. per liter of nutrient. The growth substances, vitamin B₁ (Merck) and naphthaleneacetic acid, were mixed with the nutrient solution immediately before it was supplied to the plant, each being prepared in aqueous stock solution and used in the dilution of 0.1 mg. per liter of nutrient.

The chrysanthemum plants were harvested on July 10. The roots were separated from the tops at the soil line, and the roots were washed free of adhering sand by dipping them in salt water and then rinsing in distilled water. After being blotted to remove the excess water, they were placed in open paper containers and dried to constant weight in a draft oven. Weights were taken immediately after the plants cooled.

COSMOS AND MARIGOLD

Plants used in this more extensive investigation were chosen because they grow well under the variable conditions of temperature and light which prevail in the Chicago area during the summer months. Both the Harmony marigold and the Burpee Golden cosmos have a comparatively short vegetative period before flowering. And this variety of cosmos has been used in previous experiments of similar nature.

Four-inch glazed pots like those of the previous experiment were used. Quartz sand which had been washed and sifted to free it of organic matter was used. On June 6, selected seeds secured from the Burpee Company of Philadelphia were planted, four or five in each pot at uniform depth. One hundred thirty pots were planted for each of the four treatments of both varieties of plants used, making 1040 pots in all. They were placed at random in rows of ten each on two greenhouse benches.

Germination was almost 100 per cent. Soon after emergence, all but two seedlings were removed from each pot. Within 2 weeks the second seedling was removed, leaving only one in each pot. At this stage, plants of both varieties were very uniform in height and vigor. Complete nutrient solution was supplied twice during the first week after emergence. Beginning on the tenth day, and continuing throughout the course of the experiment, growth substances were added to the nutrient solution and supplied to the plants twice a week. Plants of one group served as controls and received only the complete nutrient solution; plants of another group received naphthaleneacetic acid added to the nutrient solution; a third group received nutrient plus nicotinic acid; and a fourth group received both nicotinic acid and vitamin B₁ in addition to the nutrient solution. All the growth substances were used in the dilution of 0.01 mg. to 1 liter of nutrient. Both marigolds and cosmos were harvested after 8 weeks. The methods used in harvesting and drying were the same as those previously described.

Results and discussion

All chrysanthemum plants of group 1 (table 1), whether or not they were supplied with growth substances in addition to the complete nutrient solution, showed similar vigor. They were very uniform in height and averaged approximately the same number of leaves. The plants which received minus Mg solution showed no detectable variation. All plants, regardless of growth substances, were definitely stunted and showed typical magnesium deficiency. Average dry weight of tops of plants receiving B₁ was slightly greater than that of the controls.

The only group which evidenced any marked response to the addition of growth substances was the one receiving minus K nutrient solution. All plants showed typical potassium deficiency, with browning and final abscission of the leaves. Both the plants receiving B₁ and those receiving naphthaleneacetic acid were more vigorous than the controls, although there was only a slight increase in height. The difference was most apparent in plants which received naphthaleneacetic acid. They showed a significant increase in dry weight. They tended to retain ten or twelve leaves in contrast to an average of nine in the controls, and the leaves were somewhat larger. There was also a tendency for the plants receiving naphthaleneacetic acid to produce one or more stolons. None was formed on the control plants.

The numbers of plants involved in the experiment were too small to justify definite conclusions, but their response gives evidence of the possibility that naphthaleneacetic acid may in some degree compensate for lack of potassium. As previously stated, the experiment with cosmos and marigold constituted the major portion of this investigation. Tables 2 and 3 show little response of the plants to the growth substances. The most striking effect was the marked individual variation in height among the cosmos plants. Each group, regardless of treatment, showed a variation in height from a minimum near 20 cm. to a maximum of over 90 cm. All plants seemed vigorous and healthy, regardless of height. This marked variability illustrates how much an inconstant characteristic might be erroneously attributed to some external factor, such as a growth substance, if plants were observed in small numbers.

Table 2 shows an accompanying variation in dry weight occurring within each group of cosmos plants. A statistical analysis to determine the significance of the variances indicates that the mean differences in weight occurring between each group and the control (table 4) lie well below the mean calculated as significant on the basis of 5 per cent probability. Comparison of the cosmos leaves showed a similar range of size in all groups. No variation in floral initiation among the groups was noted. At the time of harvest, 79 out of 130 control plants were in bud or blossom as compared with 74 in the naphthaleneacetic-acid group, 73 of those receiving nicotinic acid, and 77 receiving both nicotinic acid and B₁.

Growth of the marigolds was much more uniform. Approximately the same number of plants from each group showed floral initiation within 5 weeks. After the terminal flower had formed, axillary shoots developed with noticeable rapidity in all plants. Dry weights (table 5) indicate one noticeable variation: the weight

TABLE 2
BURPEE GOLDEN COSMOS

COMPLETE NUTRIENT (CONTROL)		COMPLETE NUTRIENT WITH					
		NAPHTHALENEACETIC ACID		NICOTINIC ACID		NICOTINIC ACID AND B ₁	
TOPS	ROOTS	TOPS	ROOTS	TOPS	ROOTS	TOPS	ROOTS

DRY WEIGHT (GM.) OF RANDOM ROWS OF TEN PLANTS EACH

37.7	9.8	43.2	11.3	52.0	12.7	55.4	14.4
57.5	13.5	54.3	16.5	53.5	14.3	50.8	13.6
56.9	16.5	53.9	13.5	49.9	12.8	57.3	15.3
52.0	14.2	57.0	16.7	49.2	16.1	59.6	14.7
57.3	16.7	55.5	13.9	58.0	14.8	58.4	14.2
52.8	12.7	53.0	13.5	51.2	14.0	56.5	14.8
56.2	13.4	44.8	12.2	57.3	15.9	46.4	13.3
52.2	14.4	51.6	16.3	56.8	14.1	56.8	15.1
51.8	16.6	49.4	16.7	55.7	14.0	54.4	12.8
50.9	14.4	60.8	14.3	55.6	16.0	55.8	15.0
63.8	15.3	51.8	13.1	62.7	17.0	63.5	17.5
59.0	15.3	52.2	13.5	49.5	13.8	40.0	10.0
39.6	12.2	38.8	11.0	44.6	11.0	50.6	13.0

AVERAGE DRY WEIGHT PER ROW OF TEN PLANTS

52.9	14.23	51.2	14.04	53.54	14.3	54.3	14.1
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AVERAGE DRY WEIGHT PER PLANT

5.29	1.423	5.12	1.404	5.354	1.43	5.43	1.41
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of the total marigold plant, and more definitely the weight of the tops of the plants receiving naphthaleneacetic acid (0.01 mg. per liter of nutrient), have less dry weight than the controls. This variance indicates that the weight lies above the calculated figure of significance at 5 per cent probability. This decreased dry weight illustrates the fact mentioned earlier, that although naphthaleneacetic acid is known to initiate root growth from stems, it may also retard growth as a result of toxic action and thus affect general plant growth.

TABLE 3
HARMONY MARIGOLD

COMPLETE NUTRIENT		COMPLETE NUTRIENT WITH					
		NAPHTHALENEACETIC ACID		NICOTINIC ACID		NICOTINIC ACID AND B ₁	
Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots
DRY WEIGHT (GM.) OF RANDOM ROWS OF TEN PLANTS EACH							
35.6	10.0	32.0	8.6	38.6	10.6	37.0	9.4
31.9	8.6	29.5	9.2	36.6	9.6	38.1	9.7
38.9	8.5	29.9	8.8	3.9	9.3	35.0	9.7
33.7	8.3	31.5	9.1	36.0	8.0	38.0	10.2
32.3	8.6	29.7	9.6	36.9	10.7	36.4	10.8
35.0	10.1	28.1	8.0	36.1	9.7	33.6	11.2
37.0	10.9	32.6	9.8	36.0	10.9	36.0	11.2
39.2	11.8	29.7	9.6	38.0	9.0	38.2	9.5
36.5	11.7	32.9	9.6	38.9	10.9	38.3	10.0
38.5	10.0	37.4	10.7	41.5	12.3	37.8	11.0
40.4	12.4	36.0	11.2	41.2	12.8	42.0	10.2
41.4	13.2	37.1	11.6	43.8	12.6	44.9	13.3
41.3	10.9	35.5	10.5	35.9	9.7	40.2	11.0
AVERAGE DRY WEIGHT PER ROW OF TEN PLANTS							
37.05	10.4	32.45	9.7	38.3	10.5	38.1	10.6
AVERAGE DRY WEIGHT PER PLANT							
3.705	1.04	3.245	0.97	3.83	1.05	3.81	1.06

TABLE 4

MEAN DRY WEIGHT IN GRAMS OF THIRTEEN ROWS OF COSMOS PLANTS USED AS CONTROLS COMPARED WITH SAME UNDER TREATMENTS INDICATED; ALSO ESTIMATED SIGNIFICANT DIFFERENCES IN WEIGHT BETWEEN CONTROLS AND TREATED PLANTS

	I CONTROL	II NAPHTHA- LENE- ACETIC ACID	SIGNIFI- CANT* DIFFER- ENCE (I AND II)	III NICOTINIC ACID	SIGNIFI- CANT* DIFFER- ENCE (I AND III)	IV NICOTINIC ACID AND B ₁	SIGNIFI- CANT* DIFFER- ENCE (I AND IV)
Tops.....	52.9	51.2	5.43	53.5	4.99	54.3	5.44
Roots.....	14.2	14.0	1.69	14.3	1.47	14.1	1.50
Total plant...	67.1	65.2	6.57	67.8	6.12	68.4	6.68

*Significant differences are weights above which, at probabilities of 5 per cent, mean differences may be considered statistically significant.

The dry weights of marigold plants supplied with nicotinic acid, and with nicotinic acid and vitamin B₁ combined, showed no increased growth response. Obviously, although vitamin B₁ and nicotinic acid are necessary for root growth, the supply produced by this plant is adequate for its needs. Consequently the external application of such substances, at least in the concentration used, has neither positive nor negative effect upon total plant growth.

TABLE 5

MEAN DRY WEIGHT IN GRAMS OF THIRTEEN ROWS OF MARIGOLD PLANTS USED AS CONTROLS COMPARED WITH SAME UNDER TREATMENTS INDICATED; ALSO ESTIMATED SIGNIFICANT DIFFERENCES IN WEIGHT BETWEEN CONTROLS AND TREATED PLANTS

	I CONTROL	II NAPHTHA- LENE- ACETIC ACID	SIGNIFI- CANT* DIFFER- ENCE (I AND II)	III NICOTINIC ACID	SIGNIFI- CANT* DIFFER- ENCE (I AND III)	IV NICOTINIC ACID AND B ₁	SIGNIFI- CANT* DIFFER- ENCE (I AND IV)
Tops.....	37.0	32.4†	2.58	38.3	2.33	38.1	2.50
Roots.....	10.4	9.7	1.09	10.5	1.23	10.6	1.09
Total plant...	47.4	42.1†	3.48	48.8	3.36	48.7	3.30

* Significant differences are weights above which, at probabilities of 5 per cent, mean differences may be considered statistically significant.

† Weights indicating statistical significance.

Summary

1. Neither vitamin B₁ nor naphthaleneacetic acid supplied to chrysanthemum plants in a concentration of 0.1 mg. per liter of complete nutrient solution produced any apparent growth responses or significant difference in dry weight.
2. Chrysanthemum plants receiving naphthaleneacetic acid in a concentration of 0.1 mg. per liter of minus K nutrient solution showed an increase in dry weight over the controls, indicating that the acid can compensate to some extent for the lack of potassium.
3. No significant difference in dry weight occurred between cosmos plants used as controls and those receiving 0.01 mg. of naphthaleneacetic acid, nicotinic acid, or nicotinic acid and vitamin B₁ per liter of complete nutrient.
4. Marigold plants showed little variation in dry weight when supplied with nicotinic acid, or with a combination of nicotinic acid and vitamin B₁.
5. A significant decrease in total dry weight of the marigold plants supplied with naphthaleneacetic acid (0.01 mg. per liter) indicates a retarding effect of the acid on growth.
6. None of the treatments to which the cosmos and marigold plants were subjected resulted in visible effects upon their habit of growth or upon floral initiation.

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